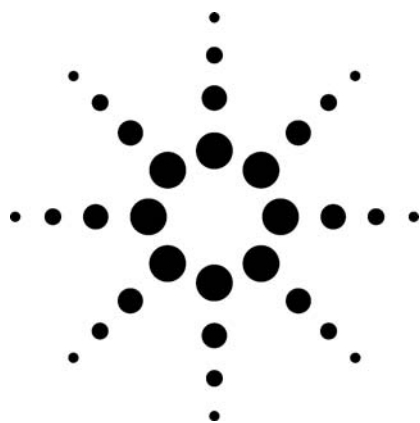# Quantitative Analysis of Perfluorooctanoic Acid by LC/MS/MS

Application Brief

Environmental

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Abstract

An Agilent 6410 Triple Quadrupole Mass Spectrometer (QQQ) is used to analyze perfluorooctanoic acid (PFOA). A simple isocratic elution is carried out on a Rapid Resolution High Throughput C18 column (particle size 1.8 µm) with only water and methanol solvents containing 10 mM ammonium acetate. Elution time for standard dilutions of PFOA is only 2.3 minutes. Good linearity over more than 4 orders of magnitude, from 9 fg/µL to 150 pg/µL, is demonstrated with excellent peak area reproducibility of 5.5 % RSD at the 9 fg/µL level. The average peak-to-peak signal-to-noise (S/N) ratio at this level is 7.2. Sensitivity of surface water extracts is expected to be similar.

Introduction

Perfluorooctanoic acid (PFOA) is a synthetic chemical that does not occur naturally in the environment.

Companies use PFOA to make fluoropolymers, which are substances with valuable properties for applications such as fire resistance and oil, stain, and grease repellence. Toxicological studies have shown that exposure to PFOA can result in developmental/reproductive toxicity, liver damage, and possibly cancer. PFOA is highly persistent in the environment and has been found at very low levels in both the environment and in the blood of the general U.S. population. Recent studies by the EPA have indicated the need for additional testing and monitoring. Detection at levels < 100 ppt in drinking water is required by the European Union.

In this work, dilutions of the PFOA standard are run at levels ranging from 9 fg/µL to 150 pg/µL, with a correlation coefficient of linearity of $R^2 > 0.997$. Nonlinearity is seen with the 1,500 pg/µL level. During the worklist the 9 fg/µL level is injected six times in a row to determine reproducibility. Based on peak areas the reproducibility at this lowest level of investigation is 5.5% RSD. Since the injection volume is 10 µL, the on-column injection amount at this lowest level is only 90 fg.

The structure of PFOA is shown below.

$$CF_3 - (CF_2)_6 - COOH$$
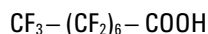
This molecule is a carboxylic acid, which is expected to show good sensitivity in negative ion mode using electrospray ionization (ESI).

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Experimental

Sample Preparation

The PFOA standard is obtained at a concentration of 1,500 ng/µL. Dilutions in methanol are made up at 0.009, 0.015, 0.15, 0.45, 0.75, 1.5, 15.0, 150, and 1,500 pg/µL concentrations.

LC/MS Method Details

LC Conditions

Agilent 1100 Series binary pump, degasser, wellplate sampler, and thermostatted column compartment

Column:	Agilent ZORBAX Eclipse Plus RRHT C18, 2.1 mm × 100 mm, 1.8 µm (p/n 959764-902)
Column temperature:	40 °C
Mobile phase:	A = 10 mM ammonium acetate in water B = 10 mM ammonium acetate in 80:20 methanol/water
Flow rate:	0.3 mL/min
Injection volume:	10 µL
Isocratic:	85% B
Stop time:	3 minutes
Needle wash:	75:25 methanol/water; flush port 10 seconds

MS Conditions

Mode:	Negative ESI using the Agilent G1948B ionization source
Nebulizer:	35 psig
Drying gas flow:	10 L/min
Drying gas temp:	300 °C
V_{cap}:	4,000 V
Resolution (FWHM):	Q1 = 0.7 amu; Q2 = 0.7 amu
MRM transition:	m/z 413.0 > 369.0
Fragmentor:	67 V
Collision energy:	3 V
Dwell time:	200 msec

Results and Discussion

The calibration curve for this work is shown in Figure 1. At the lowest level of investigation (9 fg/µL) nine replicate injections are made. Nine replicate injections are also made at the 15 fg/µL level. As seen in Figure 2, the peak area reproducibility is 5.5% RSD. The average S/N ratio at this level is 7.2. Noise is calculated from the 1– to 1.5–minute region.
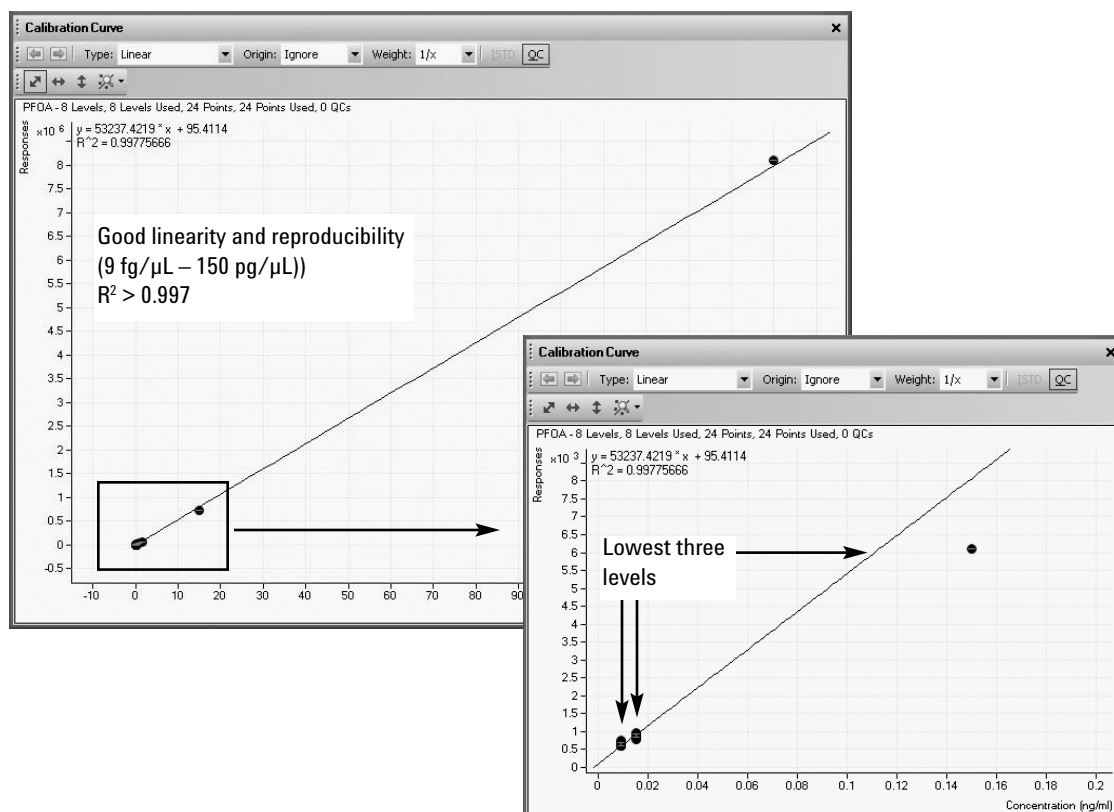

Figure 1. Calibration curve for PFOA. Lowest three levels consist of nine replicate injections at 9 fg/µL and 15 fg/µL each, and one injection at 150 fg/µL.
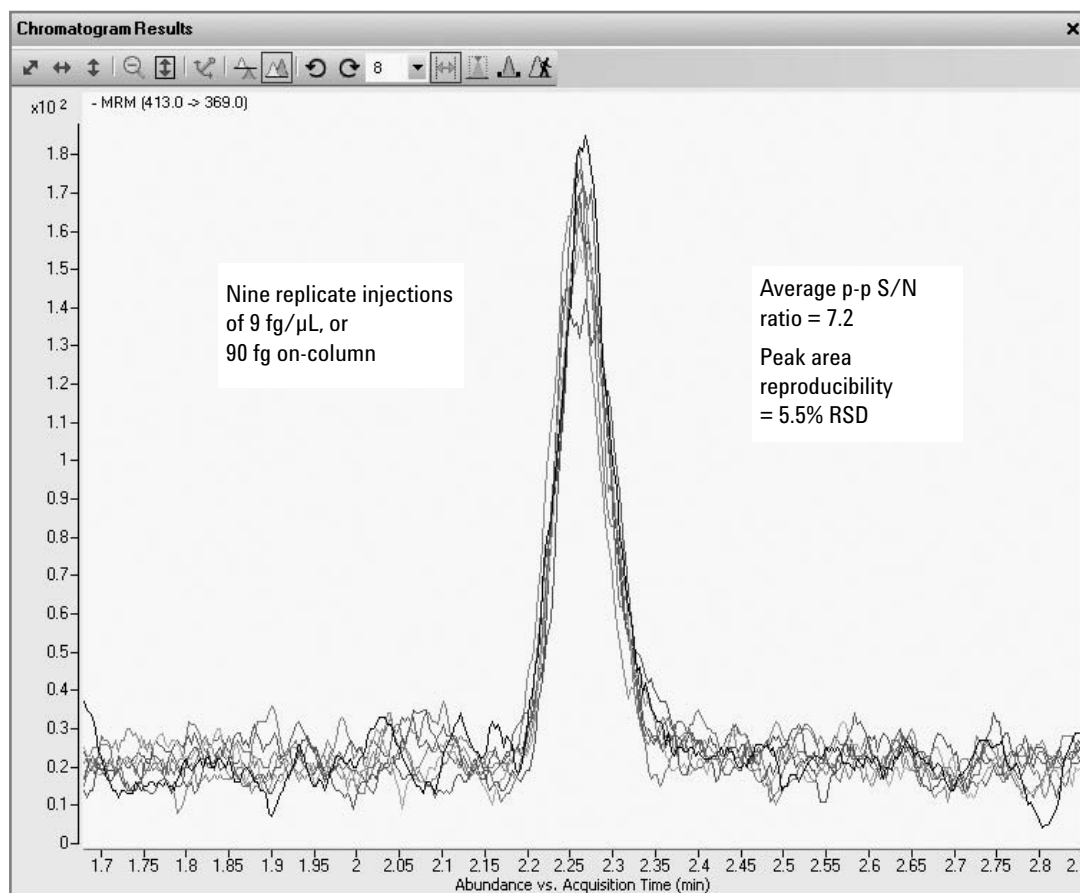
Figure 2.   Excellent sensitivity and reproducibility at the lowest level investigated.

If an additional dilution level of 1,500 pg/µL is added to the calibration curve, then we see the result shown in Figure 3, in which a quadratic curve fit of the data is required. However, note the excellent correlation coefficient of $R^2 > 0.99999$.

Conclusions

The perfluorooctanoic acid compound is very sensitive to negative electrospray ionization mode. Very good linearity is demonstrated with the standard at dilutions ranging from 9 fg/uL to 150 pg/µL, which corresponds to an on-column injection amount ranging from 90 fg to 1.5 ng. Peak area reproducibility at the lowest level investigated of 9 fg/µL is excellent at 5.5% RSD, and the average signal-to-noise ratio of the replicate injections at this level is 7.2. Signal saturation is seen at 1,500 pg/µL, or 15 ng on-column. As drinking water is a relatively clean matrix, it is expected that similar sensitivity will be achievable with extracted samples.

For More Information

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For more details concerning this application brief, please contact Michael Zumwalt at Agilent Technologies, Inc
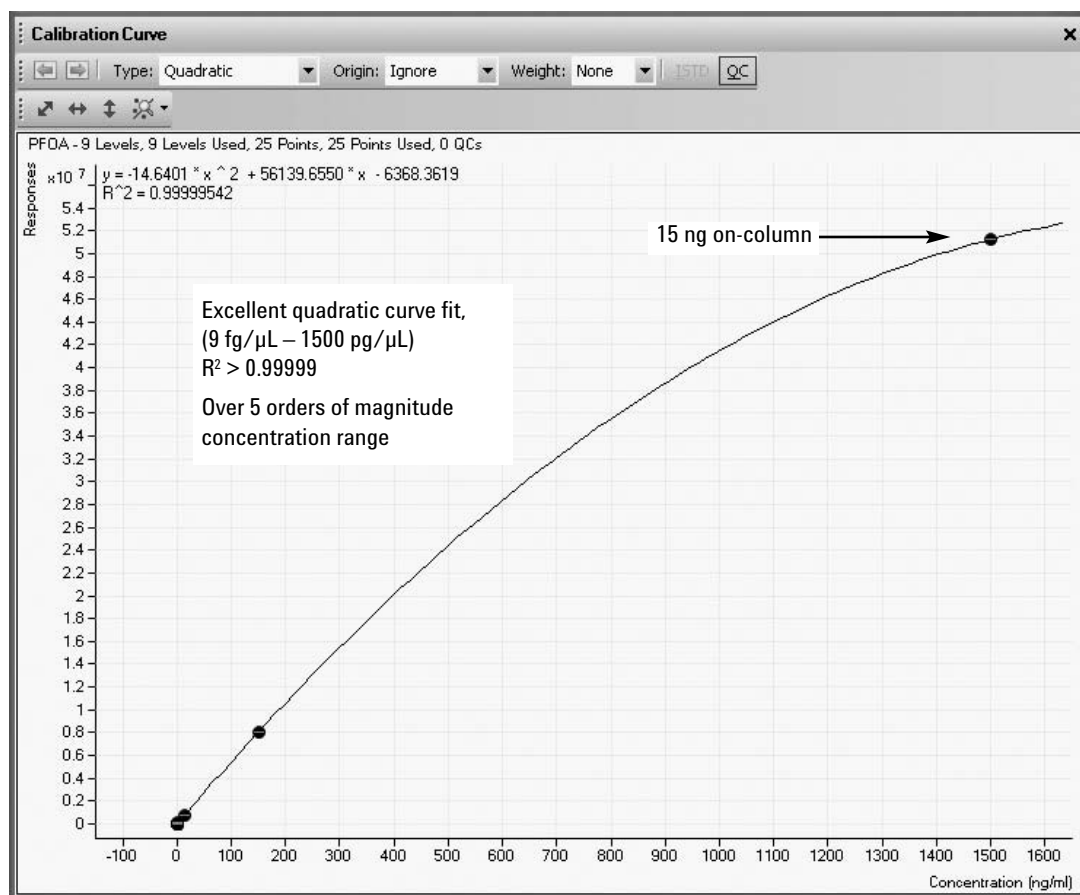
Calibration Curve

Type: Quadratic   Origin: Ignore   Weight: None   ISTD QC

PFOA - 9 Levels, 9 Levels Used, 25 Points, 25 Points Used, 0 QCs

$y = -14.6401 * x^2 + 56139.6550 * x - 6368.3619$
$R^2 = 0.99999542$

15 ng on-column →

Excellent quadratic curve fit,
(9 fg/µL – 1500 pg/µL)
$R^2 > 0.99999$

Over 5 orders of magnitude
concentration range

Figure 3.   **Saturation seen with addition of 1500 pg/µL level, or 15 ng on-column.**

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